

## Cell Line Nucleofector™ Kit R

### for NIH/3T3 Cells [DSMZ]

#### Product description

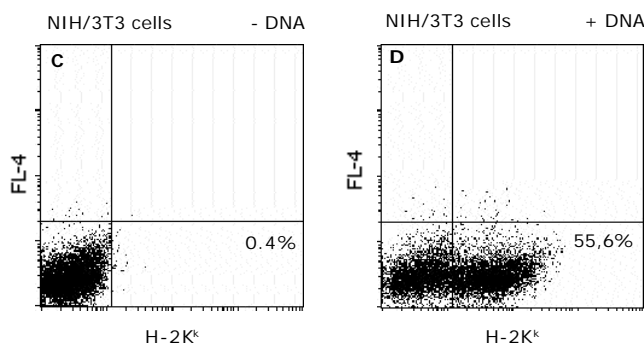
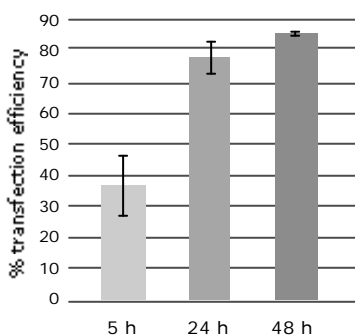
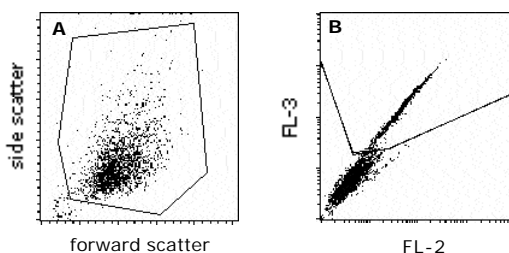
Cat. No.	<b>VCA-1001</b>
Kit components	2.25 ml <b>Cell Line Nucleofector™ Solution R</b> 0.5 ml Supplement 25 certified cuvettes 25 plastic pipettes
Size	25 reactions
Storage and stability	Store Nucleofector™ Solution and Supplement at 4°C. The expiry date is printed on the Solution Box.

#### Cell type

Origin Swiss mouse embryo (NIH/3T3) [DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; Cat. No. ACC59].

Morphology Fibroblastoid cells.

#### Nucleofector™ program **A-24**



#### Average transfection efficiency of NIH/3T3 cells.

Cells were nucleofected with program A-24 and 3 µg of plasmid encoding the enhanced green fluorescent protein eGFP. **5, 24 and 48 hours** post nucleofection, the cells were analyzed by fluorescent microscopy.

#### Example for nucleofection of NIH-3T3 cells with H-2K<sup>k</sup> cDNA.

NIH-3T3 cells were nucleofected using the Cell Line Nucleofector™ Kit R, program A-24 and a plasmid encoding the mouse MHC class I heavy chain molecule H-2K<sup>k</sup>. **4 hours** post nucleofection, the cells were stained with a Cy5-coupled antibody directed against H-2K<sup>k</sup> and were analyzed by flow cytometry. NIH-3T3 cells were gated according to forward/side scatter (A). Dead cells were excluded by staining with propidium iodide and gating (B). H-2K<sup>k</sup> expression of NIH-3T3 is shown without (C) and with plasmid DNA (D).

## Cell culture

Medium	90% Dulbecco's modified Eagle medium (DMEM) [Invitrogen/Gibco; Cat. No. 41966-029] with GlutaMAX [Invitrogen/Gibco; Cat. No. 35050-038] and 10% FCS.
Trypsin treatment	0.5 mg/ml Trypsin; 0.2 mg/ml EDTA in PBS
Passage interval	Cells should be passaged at 70-80 % confluency. The cell layer should not become completely confluent.
Seeding conditions	2.5x10 <sup>5</sup> cells per 25 cm <sup>2</sup> flask.

## Protocol

Culture conditions before nucleofection	<ul style="list-style-type: none"><li>&gt; The cells should be preferably passaged <b>at least 1 day</b> before nucleofection.</li><li>&gt; Cells should be nucleofected after reaching <b>70-80%</b> confluency. Higher cell densities may cause lower nucleofection efficiencies.</li></ul>
DNA quality	The quality and the concentration of DNA used for nucleofection plays a central role for the efficiency of gene transfer. We strongly recommend the use of high quality products for plasmid purification like Marligen Biosciences CONCERT™ High Purity Plasmid Prep Purification System or QIAGEN® EndoFree® Plasmid Kits. The purified DNA should be resuspended in deionised water or TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0) with a concentration between 1-5 µg/µl. Please check the purity of each plasmid preparation by measurement of the A260:A280 ratio. The ratio should be at least 1.8 for nucleofection.
Positive control	The easiest way to establish the Nucleofection™ technology is to use the enhanced green fluorescent protein (eGFP) for your first experiments. We strongly recommend using eGFP-plasmids like pEGFP-C1 [BD Clontech; Cat.No. 6084-1] or pEYFP-C1 [BD Clontech; Cat.No. 6005-1]. Please do not use eGFP plasmids with IRES sequences for your first experiments. We also propose using the eGFP plasmid as a positive control for all following experiments.
Preparation of the Nucleofector™ Solution	> Add 0.5 ml Supplement to 2.25 ml Nucleofector™ Solution and mix gently. The Nucleofector™ Solution is now ready to use and is stable for 3 months at 4°C. <b>Note date of addition on the vial.</b>

Nucleofection protocol:

**One nucleofection sample contains:**

- > **1x10<sup>6</sup> cells\***
- > **1-5 µg plasmid DNA (in 1-5 µl H<sub>2</sub>O or TE)**
- > **100 µl Nucleofector™ Solution R**

\* Minimal recommended cell number is 5x10<sup>5</sup> cells per nucleofection sample (a lower cell number leads to a major increase in cell mortality).

1. Cultivate the required number of cells.
2. Prepare 1-5 µg DNA (in **1-5 µl** H<sub>2</sub>O or TE) for each sample.
3. Pre-warm the supplemented Nucleofector™ Solution R to room temperature. Pre-warm an aliquot of culture medium at 37°C in a 50 ml tube (500 µl per sample).
4. Prepare 6 well plates by filling appropriate number of wells with 1 ml of culture medium containing supplements and serum. Pre-incubate plates in a humidified 37°C/5% CO<sub>2</sub> incubator.
5. Remove the medium from the cell culture. Wash cells once with PBS.
6. Harvest the cells, e.g. with trypsin/EDTA and stop the trypsinization with supplemented culture medium or PBS/0.5% BSA (see Nucleofector™ Manual, section 3.6.1).
7. Take an aliquot of trypsinized cell suspension and count the cells to determine the cell density.
8. Centrifuge the required number of cells (1x10<sup>6</sup> cells per nucleofection sample) at 200xg for 10 min. Discard supernatant completely so that no residual medium covers the cell pellet.
9. Resuspend the pellet in room temperature Nucleofector™ Solution R to a final concentration of 1x10<sup>6</sup> cells/100 µl. Avoid storing the cell suspension longer than 15 min in Nucleofection™ Solution, as this reduces cell viability and gene transfer efficiency.  
**Important: Steps 10-14 should be performed for each sample separately.**
10. Mix 100 µl of cell suspension (see step 9) with 1-5 µg DNA (in 1-5 µl H<sub>2</sub>O or TE).
11. Transfer the nucleofection sample into an amaxa certified cuvette. Make sure that the sample covers the bottom of the cuvette, avoid air bubbles while pipetting. Close the cuvette with the blue cap.
12. Insert the cuvette into the cuvette holder and rotate the turning wheel clockwise to the final position. Select the program **A-24** (see Nucleofector™ Manual, section 2.6). Press the "X" key to start the program.
13. **To avoid damage to the cells remove the sample from the cuvette immediately after the program has finished** (display showing "OK"). Take the cuvette out of the holder. To transfer the cells from the cuvettes, we strongly recommend using the plastic pipettes provided in the kit to prevent damage and loss of cells.

Add 500 µl of pre-warmed **RPMI supplemented with 10% serum** and transfer the sample into the prepared 6 well plates. Alternatively, transfer the sample into a 1.5 ml microcentrifuge tube and place it in a 37°C heat block.

14. Press any key to reset the Nucleofector™.
15. Repeat steps 10-14 for the remaining samples.
16. If you have incubated the samples in 1.5 ml microcentrifuge tubes transfer them into the prepared 6-well plates.
17. Incubate cells in a humidified 37°C/5% CO<sub>2</sub> incubator. After 4 hours of incubation viability of cells can be evaluated by proportion of cells attached to the culture wells. Depending on the gene, expression is often detectable after 4-8 hours. If this is not the case, the incubation period may be prolonged up to 24 hours.